Evoked assay of neural circuit function with microelectrode array (MEA) technology and *in vitro* cell culture models

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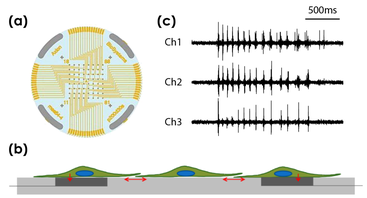
Multiwell MEA Technology

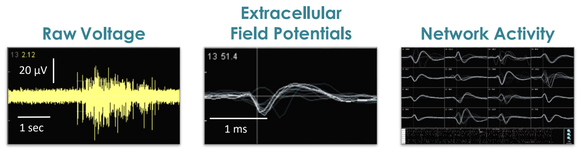
Why Use Microelectrode Arrays?

Microelectrode arrays (MEAs) monitor and manipulate cultured cell activity *in vitro*, providing insight into neural networks to inform disease-in-a-dish models, stem cell characterization, and drug development. Axion BioSystems' Maestro™ multiwell MEA platforms enable high-throughput assessment of neural networks at reduced time and cost.

Optogenetics can further enhance neural assays by providing artifact-free, precise, and targeted stimulation. Here, we evaluate the application of the Lumos, a commercial multiwell optical stimulation system, and next generation opsins for *in vitro* neural assays.



A planar grid of microelectrodes **(a)** interfaces with cultured neurons or cardiomyocytes **(b)**, to model complex, human systems. Electrodes detect changes in raw voltage **(c)** and record extracellular field potentials.



Raw voltage signals are processed in real-time to obtain extracellular field potentials from across the network, providing a valuable electrophysiological phenotype for applications in disease models, drug discovery, toxicological and safety screening, and stem cell characterization.

Why Use the Maestro Pro™ and Maestro Edge™?

Axion's Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data quality and ease of use.

- **Label-free, non-invasive recording** of extracellular voltage from cultured electroactive cells
- **Integrated environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the depolarization waveform
- **Sensitive voltage resolution** detects subtle extracellular action potential events
- **Industry-leading array density** provides high quality data from across the entire culture
- **Scalable format (6-, 24-, 48- and 96-well plates)** meets all throughput needs on a single system
- **State-of-the-art electrode processing chip (BioCore v4)** offers stronger signals, ultra-low frequency content, and enhanced flexibility

Optogenetics to Control Complex Biology

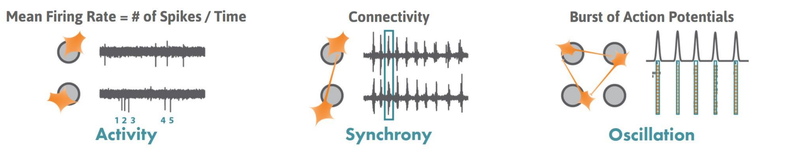
Optogenetics is the integration of fast, light-activated ion channels (opsins) to enable targeted manipulation of cell activity or intracellular signaling. Optogenetic techniques enable:

- Artifact-free stimulation for pacing cardiomyocytes or controlling neural activity
- Bi-directional control of activity via activation or inhibition of cell subtypes
- Genetic targeting for cell type specificity
- Control of gene expression and intracellular signaling for enhanced development of disease-in-a-dish models
- Establishing well-to-well and assay-to-assay consistency for more reliable results

MEA Assay with Neurons

Neural Electrophysiology Phenotypes

AxIS Navigator™ and analysis software provide intuitive measures of neuron and network function.



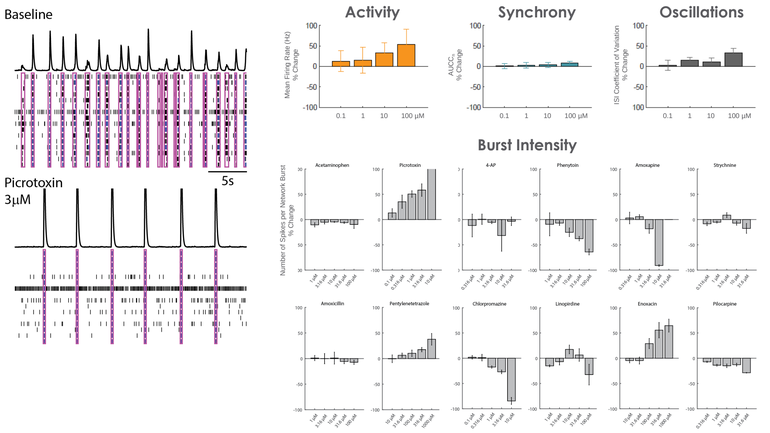
Are my neurons functional?
Action potentials are the defining feature of neuron function. High values indicate the neurons are firing action potentials frequently. Low values indicate the neurons may have impaired electrophysiological function.

Are my synapses functional?
Synapses are functional connections between neurons, such that an action potential from one neuron affects the likelihood of an action potential from another neuron. Synchrony reflects the strength of synaptic connections.

Is my network functional?
Neural oscillations, defined by alternating periods of high and low activity, are a hallmark of functional networks with excitatory and inhibitory neurons. Oscillation is a measure of how the network activity is organized in time.

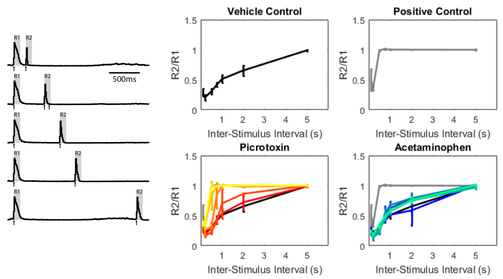
Network Electrophysiology Assays for Proconvulsant Assessment

Primary rodent cortical neurons (Thermo Fisher) were cultured on a CytoView MEA 48-Well plate. Neuronal activity, synchrony between neurons, and oscillations of network activity were assessed before and after addition of compounds at 32 days *in vitro*. At Baseline, cultures exhibited irregular network bursts characterized by periods of increased synchronous activity interspersed with periods of lower activity. After addition of picrotoxin, activity and oscillations increased with increasing doses of picrotoxin. This increase in oscillations was also characterized by an increase in both the strength of the network bursts as well as the quiescence between bursts.



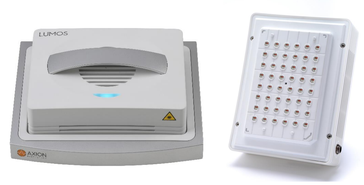
Evoked Neural Activity for Seizurogenic Screening

Stimulation enables the computation of evoked activity measures. For each electrode, and each well, key parameters of the stimulus-evoked response can be calculated and used to inform assessment of seizurogenic activity. As an example, a "paired stimulus" assay can assess the excitatory-inhibitory balance of a network. The ratio of the response for two stimuli, with increasing delay between them, is highly sensitive to compounds, like picrotoxin, that decrease inhibition in the network.



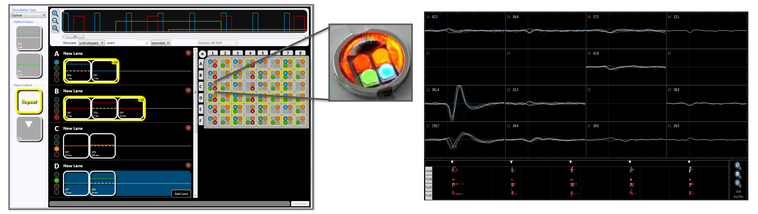
Multiwell Optical Control

Why Use the Lumos™?



The Lumos™ is the first commercial multiwell light delivery device designed for *in vitro* optogenetics. The Lumos provides precise control over cardiomyocyte beat rate or neural activity.

- **Artifact-free stimulation and pacing**
- **High throughput** with 192 LEDs over 48, 24 or, 96 wells
- **Compatible with any opsin** with 4 wavelengths encompassing the visual spectrum (460-670 nm)
- **Maximal intensity** with high power LEDs and optimized plate and lid optics on the Lumos MEA
- **Precise control** with microsecond precision and finely adjustable intensity for each LED
- **Flexible control** as each LED can be controlled independently and simultaneously

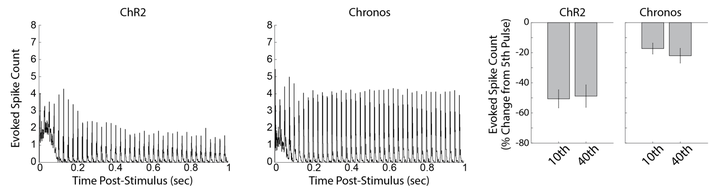


AxIS Stimulation Studio offers intuitive stimulus design with drag and drop blocks to create light delivery patterns and clickable selection of target wells. Stimulus responses are visualized in real time for easy interpretation.

High Frequency Optical Stimulation

Chronos Enables High Frequency Responses without Adaptation

High frequency blue light stimulation (2ms pulses at 40Hz for 1s) was applied with the Lumos to ChR2+ primary rat neurons. The evoked neural response showed adaptation with reduced responses to later pulses in the train. In contrast, Chronos' faster kinetics enabled a consistent response to each pulse in the high frequency train.



High Frequency Stimulation to Probe Excitatory-Inhibitory Balance

ChR2+ and Chronos+ neurons were dosed with either a proconvulsant (Picrotoxin, a $GABA_AR$ antagonist,10µM), antiepileptic (Carbamazepine, a sodium channel blocker, 30µM), or vehicle control (DMSO). Neural networks dosed with Picrotoxin showed a sustained response to high frequency blue light stimulation (2ms pulses at 40Hz for 1s) in which firing did not return to zero between each pulse, resulting in a higher cumulative spike count across the 40 pulses (right). In contrast, carbamazepine reduced the response, resulting in a lower cumulative spike count.